A Detailed Kinetic Model of Immunoreceptor Signaling

Development and Preliminary Analysis

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ABSTRACT

The "cell signaling problem" is under intense experimental study. Ongoing efforts have identified many of the components of specific signaling pathways (receptors, enzymes, adapters, etc.) and, for each, determined which molecules it interacts with, how it is regulated and what functions it performs. As the number of identified molecules has grown and new regulation mechanisms have been discovered, it has become clear that a major problem will be how to incorporate this information into a useful predictive model. One receptor system that has received considerable attention is the multisubunit immune recognition receptor, FcϵRI, that plays a central role in allergic reactions of the immediate type [1–3]. On the surface of basophils and mast cells FcϵRI is complexed with IgE. A signaling cascade is initiated when a multivalent antigen binds to IgE and causes IgE-FcϵRI complexes to form aggregates. Aggregation is followed rapidly by phosphorylation of tyrosines on receptor subunits, which facilitates binding of additional protein kinases and regulator molecules that comprise the signaling cascade.

We have developed a detailed kinetic model of the early signaling events that occur after ligand-induced aggregation of FcϵRI. Our model allows for dimerization of ligand-receptor complexes and includes two receptor subunits that upon phosphorylation become binding sites for protein kinases. The model includes two kinases, Lyn, which is attached to the inner leaflet of the cell membrane, and Syk, which is present in the cytosol and possesses several additional tyrosines that when phosphorylated regulate its activity and control docking of additional signaling molecules. The aggregation of receptors and the number of different phosphorylation patterns create enormous complexity in this system. In our model, which is highly simplified compared with the actual FcϵRI receptor system because we lump together phosphorylation sites on each receptor subunit into a single site, there are 354 unique chemical species and over 10,000 possible reactions among them. We estimate that more complete models will contain on the order of 10,000 species joined by millions of possible reactions. Simply encoding such a network in an error-free way is a challenging task. In order to generate the states and chemical rate equations in our network, we have developed a robust labeling system for receptor states and used a tree algorithm to generate all of the possible states. The possible chemical transformations are then enumerated by applying a set of fundamental reaction rules.

To obtain the time courses and steady-state values of the species in the reaction network generated by our model, we have taken two approaches. The first is to generate a series of rate equations for the concentration of each chemical species in the model and solve these as a system of coupled differential equations using standard techniques. The second method we have applied is the kinetic Monte Carlo approach pioneered by Gillespie [4], which treats each of the molecular species in the system as a discrete entity. There are two primary advantages of the latter approach. The first is that the stochastic approach yields not only the average number or concentration of a given species in the system, but also the fluctuations about that average, which may be important when the average value is small. Second, and more important for our model, is that the stochastic approach allows us to trace the path of a given molecule through the network, or to determine the probability of a given sequence of reactions. Through these types of analyses we hope to identify candidate reactions or reaction sequences as targets for therapeutic intervention.

One motivation for developing such detailed models is to aid the development of highly specific drugs to suppress undesirable responses. A more immediate goal is the development of a predictive model that can be used to assist in ongoing experimental efforts to map out the FcϵRI receptor pathway using genetically engineered cell lines. H. Metzger and coworkers at NIH have developed stable transfectants that express a small subset of the signaling molecules. Data from these experiments have been used to determine the parameter values in our model, and the model has in turn been used to suggest new experiments that further map out the pathways or test hypothesized relationships between ligand-receptor binding properties and the activation of signaling molecules.

REFERENCES

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